EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Synergistic interactions between small unilamellar vesicles composed of DMPC with the NSAID Ibuprofen and the Saponin Glycyrrhizin: A combined SAXS/WAXS study	**Experiment number**: SC5102
Beamline: ID02	**Date of experiment**: from: 21.04.2021 to: 24.04.2021	**Date of report**: 19.08.2021
Shifts: 9	**Local contact(s)**: Thomas Zinn, Lauren Matthews	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The interaction of the saponin aescin and ibuprofen in small lamellar vesicles (SUVs) as model membranes was already studied in our previous work.[1] Aescin and ibuprofen have a synergistic effect on the structural membrane parameters. During this beamtime, we investigated a similar effect for mixtures of glycyrrhizin and ibuprofen in DMPC model membranes.

The aim of this experiment was to resolve the interaction of the nonsteroidal anti-inflammatory drug ibuprofen and the saponin glycyrrhizin with DMPC model membranes in the form of unilamellar vesicles. We expected to resolve the effect of ibuprofen and glycyrrhizin content on the temperature dependent structural changes and on the main phase transition of the lipid.

This experiment was a remote experiment due to the Covid-19 pandemic. The samples pre-prepared were sent to ESRF a few days in advance. Samples containing DMPC (15 mg/mL), ibuprofen (5, 10 mol%) and glycyrrhizin (5, 7, 10, 50, 60, 65, 70, 75, 80, 85, 90 mol%) in a phosphate buffer (pH = 7.4) were measured by the local contacts. The pure DMPC vesicles and samples containing 5, 7 and 10 mol% glycyrrhizin were extruded through a membrane with a pore size of 500 Å three days prior to the beamtime. All other samples were measured without extrusion. Measurements were performed in a temperature controlled flow-through capillary (quartz, inner diameter 2 mm) in a temperature range of 10 to 50 °C in steps of 5 °C. Sample-to-detector distances of 1.3 and 10 m were used, simultaneously the WAXS signal (sample-to-detector distance of 0.1 m) was detected.

Figure 1 shows exemplarily the normalized, averaged, background subtracted and rebined dataset for DMPC vesicles with glycyrrhizin (10 mol%) and ibuprofen (10 mol%, left; 5 mol%, right) in a temperature range of 10 to 45 °C. The curves are scaled by multiples of 10. At low *q*-values the curves show the typical form factor of vesicles. The radius of the vesicles increases with increasing temperature. In the temperature range around the main phase transition temperature of DMPC (23.6 °C) a structural change is observed, caused by melting

of the DMPC acyl chains. At higher *q*-values we obtain information about the local bilayer structure. Here, the bilayer structure changes with increasing temperature. The significant change is observed around 25 °C.

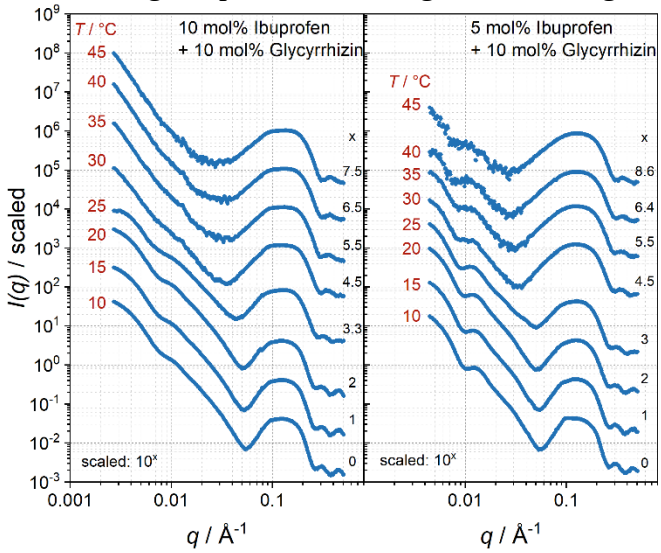

Fig. 1 Scattering curves of DMPC vesicles with glycyrrhizin (10 mol%) and ibuprofen (10 mol%, left; 5 mol%, right) at different temperatures (10 – 45 °C). The curves are scaled with a factor x indicated on the right of the respective curve.

At higher content of the saponin the system is known to form bicelles [2]. The present experiments reveal this bicelle formation also in presence of the drug. Figure 2 shows exemplarily the normalized, averaged, background subtracted and rebined dataset for such DMPC bicelles with glycyrrhizin (70 mol%) and ibuprofen (10 mol%, left; 5 mol%, right) in a temperature range of 10 to 45 °C. The curves are again scaled by multiples of 10.This is also confirmed by the shape of the scattering curves (Fig. 2). We observe changes in the scattering curves at low *q*-values which is due to the change of the global size and shape of the bicelles.

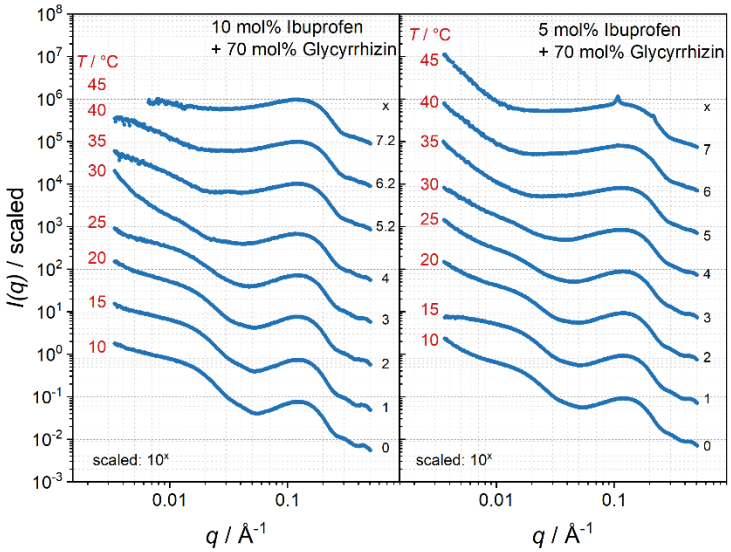

Fig. 2 Scattering curves of DMPC bicelles with glycyrrhizin (70 mol%) and ibuprofen (10 mol%, left; 5 mol%, right) at different temperatures (10 – 45 °C). The curves are scaled with a factor x indicated on the right of the respective curve.

References:

[1] R. Sreij et al., *Interaction of the Saponin Aescin with Ibuprofen in DMPC Model Membranes*, Mol. Pharmaceutics 15, 4446-4461, **2018**.

[2] R. Geisler et al., *Aescin – a naturural soap for the formation of lipid nandiscs with with tunable size*, Soft Matter, 17, 188-1900, **2021**.